

## BLOOD SUGAR AND ACTIVITY IN FISHES WITH NOTES ON THE ACTION OF INSULIN

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The blood sugar of fishes has been studied by numerous investigators and great variations in amount have been reported for different species. In most cases a given observer has worked on one or a very few species, and correlations between the amount of sugar and the habits of the fishes have not been attempted. Furthermore, it is difficult to compare the results of different authors, since so many methods of determining blood sugar have been employed. Macleod (1926), has suggested that the more active fishes have higher blood sugar than do the more sluggish forms. One of us (Gray, 1929), has also pointed out a correlation between activity and blood sugar, but detailed data were not given. Among the mammals, Shirley (1928) hints at a tendency for low blood sugar to accompany high activity. She, however, did not make a comparative study, but limited her observations to a single species.

In a previous paper (Hall and Gray, 1929), a correlation was pointed out between the habits of marine fishes and their hemoglobin concentration. It was shown that among fifteen species of marine teleosts, in general, the most active had the highest iron values, while the blood of sluggish fishes had low iron content. The fishes with the highest iron content were surface feeding forms with similar habits, and fishermen consider them among the fastest swimmers. The highest hemoglobin was noted among members of the families Scombridæ and Clupeidæ, examples of which are, respectively, the mackerels and menhaden. These fishes feed largely on plankton and small fishes, which they can only obtain by constantly keeping in motion. At the other extreme are the bottom feeders, such as the goosefish, toadfish, and sand dab, which are very sluggish and have extremely low hemoglobin. These forms remain quiescent on the bottom for long periods of time. Between the two extremes are found the majority of fishes.

In the present paper it is shown that correlations similar to those between hemoglobin and activity exist also between blood sugar and activity.

This work was carried on at the United States Fisheries Station at Woods Hole, Massachusetts.

## MATERIALS AND METHODS

Blood sugar determinations were made on fifteen different species of teleosts, representing thirteen families, as shown in Table I. The fishes were obtained from commercial fish traps and were carefully placed in large floating "live cages," where they were kept free from asphyxial conditions for at least twenty-four hours before use. The importance of keeping the fishes free from asphyxial conditions cannot be overemphasized. In a previous paper (Hall, Gray, and Lepkovsky, 1926), the changes that take place in the concentration of the blood constituents of fishes under asphyxia were pointed out. Other workers (McCormick and Macleod, 1925; Simpson, 1926; and Menten, 1927) have noted that asphyxia tends to raise the blood sugar. The time required for fishes brought to the laboratory to recover from the partial asphyxia to which they have been subjected incidental to capture and transportation varies, of course, with the different species and with the methods of handling, both before and after they are placed in the "live cage." McCormick and MacLeod found that it required from two to four days for asphyxial hyperglycemia of the sculpin to subside. With our methods and facilities it was found that one full day was generally enough time to allow for recovery from any asphyxia to which the fishes might have been subjected. Menton (1927), concludes that the variation in sugar content of a species is governed largely by the amount of food ingested. In our experiments the food factor was reduced to a minimum by not using the fishes for a day or more after placing them in the "live cage."

The methods of procedure were similar to those employed in previous studies. The puffers, toadfish, and goosefish were bled from the heart with a hypodermic needle. The other fishes were bled by severing the tail and collecting the blood from the caudal vessels in a small Erlenmeyer flask. Lithium oxalate was used as an anti-coagulant. The blood sugar was determined by Folin's modification of the Folin-Wu method (Folin, 1926; Folin and Svedberg, 1926). A large percentage of the determinations was made on the same sample of blood used for the iron determinations (Hall and Gray, 1929), to which reference has previously been made. One fish was used for each determination.

During the study of the action of insulin the fishes were kept in hatchery boxes, one fish to each box. Insulin from Eli Lilly and Company was used throughout. The insulin was administered by intraperitoneal injections, in doses of five to fifteen units, depending on the size and species of fish. If the action of insulin in fishes is similar to its action in mammals, overdoses were given in each case.

TABLE I  
*The Blood Sugar of Marine Fishes*

Species	Family	No. of Determinations	Sugar per 100 cc. of Blood		
			Low	High	Average
			Mg.	Mg.	Mg.
Group I					
Bull's eye mackerel ( <i>Pneumatophorus colias</i> ) . . . . .	Scombridae	10	69.2	160.0	90.7
Butterfish ( <i>Poronotus triacanthus</i> ) . . . . .	Stromateidae	8	57.5	113.6	79.4
Menhaden ( <i>Brevoortia tyrannus</i> ) . . . . .	Clupeidae	30	52.9	151.5	75.2
Rudderfish ( <i>Palinurichthys perciformis</i> ) . . . . .	Centrolophidae	7	54.9	83.3	67.7
Common mackerel ( <i>Scomber scombrus</i> ) . . . . .	Scombridae	9	48.5	76.6	63.5
Eel ( <i>Anguilla rostrata</i> ) . . . . .	Anguillidae	4	40.6	67.6	59.0
Bonito ( <i>Sarda sarda</i> ) . . . . .	Scombridae	3	48.5	62.7	55.1
Scup ( <i>Stenotomus chrysops</i> ) . . . . .	Sparidae	46	35.3	81.4	52.6
Silver hake ( <i>Merluccius bilinearis</i> ) . . . . .	Merlucciidae	9	25.3	85.4	48.2
Group II					
Sea robin ( <i>Prionotus carolinus</i> ) . . . . .	Triglidae	9	20.8	60.9	37.4
Sand dab ( <i>Lophopsetta maculata</i> ) . . . . .	Pleuronectidae	4	24.6	42.5	31.0
Cunner ( <i>Tautoglabrus adspersus</i> ) . . . . .	Labridae	4	13.4	35.1	25.2
Puffer ( <i>Spheroides maculatus</i> ) . . . . .	Tetraodontidae	15	4.5	41.3	23.1
Toadfish ( <i>Opsanus tau</i> ) . . . . .	Batrachoididae	6	10.2	22.3	15.4
Goosefish ( <i>Lophius piscatorius</i> ) . . . . .	Lophiidae	11	0.0	10.3	5.6

#### RESULTS AND DISCUSSION

The results of the blood sugar determinations of the fifteen species of marine teleosts are given in Table I. The fishes were kept under conditions approximating the normal as nearly as possible. The high and the low blood sugar values are given together with the average to show the individual variation within the same species. The high and the low values may seem exceedingly far apart in a few cases, and without explanation may be misleading. The great majority of blood

sugar determinations gave results near the average; it was only occasionally that a very high or exceedingly low value was obtained. There appears to be, however, a relatively greater individual variation among fishes of the same species kept under the same conditions, than among mammals.

Blood sugar, like hemoglobin, appears to be correlated in a general way with the habits and activity of the fishes. The bull's eye mackerel, butterfish, menhaden, rudderfish, common mackerel, eel, bonito, scup, and silver hake are not only more active fishes than the others given in the table, but also have higher blood sugar. For convenience of discussion the fishes are arbitrarily divided into groups I and II. There is no sharp dividing line, however, between the two groups.

Group I consists, for the most part, of aggressive fishes that depend on their own activities in obtaining food. They feed largely on plankton, small fishes, or other small animals that require the expenditure of considerable effort to obtain. Members of the Scombridæ and Clupeidæ are especially noted for their great activity. Individuals of these families are kept in captivity only with great difficulty even when placed in large "live cages" where they have plenty of room for their constant movements. It is doubtful if the bonito, mackerels, and menhaden ever cease their movements.

Some fishes of group I, for example, the scup and hake, might well be classed as intermediate in regard to their activity. They are not always in motion, nor do they pursue their food with the aggressiveness shown by the Scombridæ and Clupeidæ.

The fishes are arranged in the table, not in the order of their activity, but according to their blood sugar content. The correlation between activity and blood sugar is not absolute but occurs in most cases. If arranged according to relative activity the bonito would be at or near the top. The blood sugar of this species may not be strictly comparable with that of the other fishes since it was impossible to keep the bonito alive in captivity. Consequently the only data obtainable were determinations made on three small specimens, bled as soon as brought from the traps.

Some of group I are excellent migrating fishes that move rapidly through the water in large schools. Mostly they are adapted for fast movement by being "stream-lined" with body-form either fusiform or laterally compressed.

In group II are the relatively inactive and sluggish fishes. In contrast to the majority of group I, these fishes are the less aggressive bottom feeders that are adapted to life on the bottom by having the body-form angular or depressed. The cunner, although having a body-

form resembling members of group I and being found in a variety of habitats, seems to prefer the rocky bottom and does not roam over wide areas in search of food.

It will be noted that the average blood sugar of the members of this group is considerably lower than that of group I. The goosfish and the toadfish are two of our most sluggish fishes and have very low blood sugar. Many determinations of the goosfish blood showed merely faint traces of sugar. The goosfish, although it feeds indiscriminately on other fishes, does not as a rule pursue its food. It is one of the anglers and attracts its prey by a lure on one of the dorsal fin-rays. All of the fishes of this group are known to remain quiet on the bottom for long periods of time, which habit is in sharp contrast to the activities of the mackerels and menhaden.

TABLE II  
*The Effect of Insulin on the Blood Sugar of Fishes*

Species	No. of Determinations	Units of Insulin Given	Time for Shock to Appear	Sugar per 100 cc. of Blood	
				Normal	After Insulin
			<i>Hours</i>	<i>Mgs.</i>	<i>Mgs.</i>
Group I					
Menhaden.....	6	5	1½- 3	75.2	8.6-20.2
Common mackerel.....	13	5	1- 4	63.5	9.4-31.2
Bull's eye mackerel.....	5	5	3- 6	90.7	9.4-11.1
Scup.....	20	5-10	10-23	52.6	0.0-15.3
Group II					
Sea robin.....	10	5-15	no shock	37.4	8.8-32.5
Puffer.....	9	5-15	no shock	23.1	0.0-13.5
Toadfish.....	15	5-15	no shock	15.4	1.5-22.9

We may say, then, that there appears to be a general correlation between the amount of sugar of the blood, the hemoglobin, the body-form, the activity, and the habits of marine fishes. Activity is expressed here qualitatively. There appears to be a dearth of quantitative determinations of metabolic activity in fishes. The oxygen consumption of the scup, puffer, and toadfish has been studied (Hall, 1929), and the results bear out our estimate of the activity of these fishes. Under the same conditions the oxygen consumption of the puffer was found to be intermediate between the relatively high consumption of the scup and the extremely low oxygen consumption of the toadfish. Because of their great activity a comparable basal oxygen consumption of the Scombridae and Clupeidae, fishes more active than the scup, could not be determined.

A further interesting relation to activity was noted through a comparative study of the action of insulin on fishes. At a temperature of 21° C. and under similar conditions, it was found that the very active fishes, menhaden, common mackerel, and bull's eye mackerel, showed insulin shock in a much shorter time than did the moderately active scup. This was perhaps to be expected. Huxley and Fulton (1924), and Olmsted (1924), have pointed out that the rate of action of insulin is dependent upon the metabolic rate of the animal itself. The more sluggish bottom feeders, sea robin, puffer, and toadfish, showed no external evidences of the effects of insulin. As has been previously noted, the normal blood sugar of these sluggish fishes is much lower than that of the more active ones. In some cases, such as the toadfish, the normal sugar concentration is not as high as the insulin-reduced sugar concentration of the more active fishes. A condensed summary of the action of insulin on fishes is given in Table II.

Insulin appears to reduce the blood sugar concentration of fishes in much the same manner as in mammals, except that a longer time is required for the action to take place. Although the number is limited, at least some of each species whose blood was analyzed showed reduced sugar concentration following insulin administration. The mackerels, menhaden, and scup, if bled during convulsions, showed reduced sugar content in each case. There is considerable individual variation in the time required for the sugar content to be reduced; and since the sluggish fishes showed no convulsions, it was difficult to estimate the length of time to allow for insulin action. Puffers, sea robins, and toadfish, bled at various intervals between twenty and forty hours after insulin injection, showed blood sugar values ranging from the normal to mere traces. Since some of each of these species showed reduction of sugar content, it is thought that in those cases where, after insulin administration, the blood sugar was within the normal range of variation, either enough time had not elapsed for the insulin to reduce the sugar concentration, or else too much time elapsed and the fishes regained the normal sugar content.

The time required for the blood sugar content to be reduced appeared to be considerably greater in these sluggish forms than in the more active fishes. It seems improbable that the failure to get insulin shock could be due to insufficient insulin. Toadfish were given repeated injections of from five to fifteen units of insulin over a period of several days with no visible signs of disturbed metabolism. With the mackerels, menhaden, or scup a single five unit injection usually resulted in death unless glucose was administered.

Insulin convulsions in fishes do not necessarily indicate that the

blood sugar concentration is reduced to its lowest level. The rate of reduction of the blood sugar values following insulin injection has been worked out for the scup and will be published later. Here it is sufficient to say that the blood sugar content may be reduced in six to eight hours in this fish. In a few cases mere traces of sugar remained in the blood after eight hours and yet in no case were convulsions apparent sooner than ten hours. In other words, a few scup had lower blood sugar before reaching the convulsive stage than did other scup in the midst of convulsions. Furthermore, the fact that sluggish fishes, as the toadfish and puffer, have their blood sugar concentration reduced without showing any shock at all, indicates that insulin shock in fishes does not have as much significance as has been attributed to insulin convulsions in mammals.

#### SUMMARY

1. Correlations between the blood sugar, hemoglobin, body-form, activity and habits of fifteen species of marine teleosts are pointed out.

2. The fishes that show the greatest activity, those that feed at the surface or are aggressively predaceous, have the highest blood sugar concentration. The sluggish bottom feeders have low sugar content in the blood.

3. Insulin shock may be easily produced in active species of fishes. In sluggish forms no external evidence of the action of insulin could be detected.

4. The blood sugar of fishes is reduced by the action of insulin. Less time is required for reduction of sugar content to take place in the active fishes than in the sluggish forms, due probably to differences in the metabolic rate of the different species. In the sluggish forms the sugar content may be reduced without convulsions or shock being apparent.

5. The normal sugar of some of the sluggish fishes is often lower than the insulin-reduced sugar of the more active fishes.